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13. ABSTRACT *(Maximum 200 words)*

The Scientific objectives of this project were the continued development of surfactant micelle and vesicle systems as reaction specific catalysts and synthetic membrane models. The principal foci of investigation were: (a) novel iodosocarboxylate catalysts for the decontamination of organphosphorus toxins and related simulants; and (b) the construction of synthetic bilayer membranes to explore the relation between lipid molecular structure and lipid dynamics within the membrane.

The final Report briefly reviews 19 publications that have been published with acknowledgements to this project grant.

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PHOSPHOROLYTIC AND MEMBRANE MIMETIC CHEMISTRY

FINAL PROGRESS REPORT (1 JUNE, 1992 - 31 MAY, 1996)

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JUNE 1, 1996

U.S. ARMY RESEARCH OFFICE

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A. Statement of the Problem Studied

The Scientific objectives of this project were the continued development of surfactant micelle and vesicle systems as reaction specific catalysts and synthetic membrane models. The principal foci of investigation were: (a) novel iodosocarboxylate catalysts for the decontamination of organophosphorus toxins and related simulants; and (b) the construction of synthetic bilayer membranes to explore the relation between lipid molecular structure and lipid dynamics within the membrane.

B. Summary of the Most Important Results*

Results obtained during the course of this project have been published in 19 technical reports; these are listed in Part C, below. Reprints of each of these reports have been furnished to ARO, so that only a very brief account will be presented here. This Summary will be divided into 2 topical areas: iodosocarboxylate reagents, and synthetic bilayer membranes or liposomes.

Iodosocarboxylate reagents. A principal theme has been the exploration of varied iodosocarboxylate structures in an attempt to maximize their reactivity toward phosphotriester simulants of organophosphorus nerve agents. Among the potent reagents developed for use in mildly basic aqueous micellar solutions of cetyltrimethylammonium chloride were a dibenzobarrelene iodosocarboxylate,[4] three isomeric iodosonaphthoates,[6] and an iodosophenanthroate.[18] Each of these reagents was several times more reactive than iodosobenzoate toward the standard simulant, p-nitrophenyldiphenyl phosphate (PNPDPP). Development of a N-hexadecylpyridinium iodosocarboxylate, with $pK_a < 5$, afforded a reagent that efficiently cleaved PNPDPP in mildly acidic solution. This extended the useful pH range of iodosocarboxylate reagents down to pH 5.[8]

The hitherto undetected phosphorylated iodosobenzoate intermediate in the iodosobenzoate cleavage of PNPDPP, was independently prepared, and could be monitored by ^{31}P NMR spectroscopy. Its very rapid destruction by even mild nucleophiles (e.g., water) could thus be verified.[12]

* Reference citations refer to the numbered publication list in Part C, below.

Modified iodosobenzoates were synthesized specifically for use in microemulsions. In particular, N-octyl-N-methyl-N, N-bis(3-carboxy-4-iodoso)benzylammonium bromide proved to be a good turnover catalyst for the cleavage of PNPDPP in a cetyltrimethylammonium bromide, N-methylpyrrolidone, toluene, and aqueous borate microemulsion.[7]

Significant reactivity enhancement in the cleavage of PNPDPP was obtained upon the covalent attachment of the PNPDPP residue to an octadecyldimethylammonium ion surfactant. Cleavages of this functional micellar substrate were carried out with iodosobenzoate, iodosonaphthoate, and copper metallomicellar catalysts.[13]

An important advance was the demonstration that O,S-dialkyl phenylphosphonothioates, simulants for the nerve agent VX, could be effectively cleaved by iodosobenzoate in micellar cetyltrimethylammonium chloride at pH 9.5. Most importantly, these reactions were chemospecific at P-S.[15]

Finally, a new initiative focused on the cleavage of phosphodiesters by lanthanide ions. We found that cleavages of a liposomal phosphodiester could be accelerated by factors of 50-70, relative to an analogous but non-aggregated phosphodiester, using europium or lanthanum-hydrogen peroxide catalysts.[16]

Liposomes and Synthetic membranes. A major concern has been the exo/endo (outside/inside) surface differentiation of synthetic liposomes by chemical means, and the dynamic processes ("flip-flop") that mediate the subsequent transfer of lipid molecules between the exo and endo leaflets of the liposomal membrane. In particular, we examined the structural features of the lipids that correlate with high or low transverse liposomal mobility. Cyclopropanated lipids, for example, were more resistant to flip-flop than the corresponding olefinic lipids; the former had fewer disruptive gauche chain conformations and were better packed.[1] On the other hand, variation of chain-to-backbone-linking functional groups showed that there was little difference in the flip-flop dynamics of ester, ether, amido, or carbamoyl-linked lipids. Flip-flop was slow at temperatures below the gel to liquid crystal transition temperature (T_c), and rapid above the T_c.[14]

Bolaamphilphilic lipids that were "stiffened" with bridging biphenyl residues, however, were quite resistant to flip-flop in surface-differentiated liposomes at temperatures significantly above the T_c. These lipids adopted extended, bilayer-bridging conformations in host liposomes.[3] Similarly, macrocyclic and bis-macrocyclic bolaamphiphilic lipids were also resistant to flip-flop in coliposomes composed of non-functional open chain host lipids.[10]

We published a review of our work on the relation of lipid molecular structure and flip-flop dynamics within bilayer membranes. The structural factors investigated included chain length, unsaturation, cyclopropanation, alkylation, "stiffening," head group type, and monopolar/bolaamphiphilic character.[11]

Modulation of lipid flip-flop by additives was also studied. The flip-flop dynamics of dipalmitoyl pseudoglyceryl ammonium ion lipids were very sensitive to added cholesterol, and this effect could be used to monitor the rate of transfer of cholesterol between cholesterol-loaded "donor" liposomes and "acceptor" liposomes.[9] The flip-flop dynamics of surface-differentiated dipalmitoylphosphatidylcholine liposomes could be modulated by an added random copolymer of (200:1) N-isopropyl- and N-octadecylacrylamide. The polymer bound to the liposomes above the polymer's lower critical solution temperature (~30°C), whereupon it underwent an extended to globular transition, which perturbed the exo leaflets of the liposomes and created defects that fostered rapid endo→exo lipid flip-flop.[5]

Very recently, we found that 7-nitro-2-oxa-1,3-diazol-4-yl (NBD)-labeled phosphatidylethanolamine was an excellent reporter of flip-flop dynamics in dipalmitoylphosphatidylcholine liposomes. The NBD probe could be used in exo-only or endo-only leaflet configurations, and thus afforded separate readouts of the dynamics of endo→exo (flip) or exo→endo (flop) lipid migrations.[17] Finally, we reported on the efficient trans→cis photoisomerization and cis→trans thermal isomerization of a pseudoglyceryl cationic azobenzene-chain lipid in hololiposomes or coliposomes. Azobenzene lipid domain formation could be followed by differential scanning calorimetry and UV spectroscopy.[19]

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D. List of All Participating Scientific Personnel

Individual	Position*	Dates on Salary	Degree Awarded
R. A. Moss	P.I.	7/1/92 - 8/31/92 7/1/93 - 8/31/93 7/1/94 - 8/31/94 7/1/95 - 8/31/95	
S. Bhattacharya	G.A.	9/1/92 - 1/31/93 2/1/94 - 8/31/94 2/1/95 - 6/30/95	
S. Bose	G.A.	7/1/94 - 8/31/94	
R. Fujiyama	P.D.	6/1/92 - 8/14/93	
W. Jiang	G.A.	7/1/94 - 1/31/95 7/1/95 - 8/31/95 2/1/96 - 5/31/96	
A. Kotchevar	P.D.	7/6/93 - 12/31/94	
G. Li	P.D.	6/1/92 - 5/31/93	
J.-M. Li	G.A.	2/1/93 - 6/30/93 9/1/93 - 1/15/94	Ph.D., 5/94
B. D. Park	P.D.	4/19/94 - 3/31/95	
H. Zhang	G.A., P.D.	7/1/92 - 8/31/94 1/16/95 - 8/31/95	Ph.D., 5/93

*G.A. = Graduate Assistant; P.D. = Postdoctoral; P.I. = Principal Investigator

E. Inventions

None